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Failure of Donor Lymphocyte Infusion to Prevent Graft Rejection in Dogs Given DLA-Identical Marrow after 1 Gy of Total Body Irradiation

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ABSTRACT

We investigated in a preclinical canine model of hematopoietic cell transplantation (HCT) whether preemptive donor lymphocyte infusion (DLI) given 1 month after HCT could prevent late graft rejection that was the rule in historical dogs given suboptimal conditioning with 1 Gy of total body irradiation (TBI) before and immunosuppression with cyclosporine (CSP) and either mycophenolate mofetil (MMF; n = 6) or rapamycin (n = 5) after dog leukocyte antigen (DLA)-identical marrow transplantation. Nine dogs given DLA-identical marrow after 1 Gy of TBI followed by postgrafting MMF and CSP were studied. A single DLI was given 28-36 days after HCT, either with (n = 5) or without (n = 4) preceding treatment with the immunosuppressive drug pentostatin. Two of the 4 dogs given DLI only maintained stable mixed donor–host chimera beyond 30 weeks after HCT, whereas 2 rejected their grafts, on weeks 10 and 15 after HCT. One of the 5 dogs given pentostatin before DLI maintained a stable mixed donor–host chimera beyond 30 weeks, whereas 4 rejected their grafts, at weeks 8, 12, 12, and 16 after HCT. The 30-week probability of stable mixed chimerism was 33% among dogs given DLI, versus 0% among 11 historical dogs (P = .003). In conclusion, DLI was only moderately effective in preventing graft rejection in this model. Additional immunosuppression with pentostatin did not improve that outcome. The model might be useful in developing potential strategies aimed at preventing graft rejection in patients with low donor chimerism levels.

KEY WORDS

Hematopoietic cell transplantation • Donor lymphocyte infusion • Pentostatin • Chimerism • Nonmyeloablative • Dog

INTRODUCTION

Nonfatal late rejections of hematopoietic grafts may occur after nonmyeloablative or reduced-intensity conditioning regimens, particularly in patients with chronic myeloid leukemia or myelodysplastic syndromes given grafts from unrelated donors [1-4]. Low levels (≤50%) of donor chimerism in T cells [5,6] and natural killer (NK) cells [1,6,7] on days 14 and 28 after transplantation are predictive of a greater risk of graft rejection. Specifically, patients with donor NK- and T-cell chimerism levels ≤50% on day 14 after hematopoietic cell transplantation (HCT) had a 50% probability of graft rejection [6]. Assessing the efficacy of strategies aimed at preventing graft rejection in patients with low-donor chimerism levels has proven difficult, because only approximately half of the patients with low donor chimerism levels eventually experience graft rejection; this emphasizes the need for a good animal model.

We have previously shown that 92% and 86% of dogs given dog leukocyte antigen (DLA)-identical marrow after 2 Gy of total body irradiation (TBI) and postgrafting immunosuppression with cyclosporine

(CSP) plus mycophenolate mofetil (MMF) [8], or with CSP plus rapamycin [9], achieved sustained mixed donor–host chimerism. This approach has been successfully translated to treat human patients with hematologic malignancies or primary immunodeficiency diseases [2,5,10], demonstrating the clinical relevance of the canine model. However, when the TBI dose was reduced to 1 Gy, graft rejection was observed in 6 of 6 dogs given CSP and MMF [8] and in 5 of 5 dogs given CSP and rapamycin [9]. Graft rejections observed after 1 Gy of TBI in this model were consistent with host-versus-graft reactions rather than with a lack of marrow space [11-13], as reviewed by Baron and Storb [10].

Here we investigated whether a preemptive donor lymphocyte infusion (DLI) given 1 month after HCT could prevent late graft rejection in dogs given 1 Gy of TBI before and MMF and CSP after DLA-identical marrow transplantation.

MATERIALS AND METHODS

Nine donor/recipient pairs (8 pairs of littermates and 1 pair of siblings), 7-10 (median 9) months old, and raised at the Fred Hutchinson Cancer Research Center, were DLA-identical on the basis of matching for highly polymorphic DLA-associated class I and class II microsatellite marker polymorphisms and DLA-DRB1 sequencing [9,14,15].

Recipients were administered 1 Gy of TBI delivered at 7 cGy/min from a linear accelerator (CLINAC 4; Varian, Palo Alto, CA). Marrow cells were aspirated from donors under general anesthesia through long needles inserted into the humeri. Marrow grafts containing 2.4×10^8-6.3×10^8 (median, 3.8×10^8) total nucleated cells per kg of the recipient were given through intravenous (IV) infusion within hours of TBI. Immunosuppression consisted of MMF, 10 mg/kg twice a day injected subcutaneously, from days 0-27, and CSP, 15 mg/kg twice a day orally, starting on day −1. CSP was discontinued either 1 day before DLI (for dogs not given pentostatin) or 1 day before pentostatin. Donor peripheral blood mononuclear cells (PBMCs) were collected 28-38 days (median, 33 days) after HCT by a COBE Spectra 6-cell separator (Grambo, Lakewood, CO) and were given through IV infusion (DLI) within hours of collection. Five dogs received posttransplantation pentostatin, 4 mg/m^2/day IV for either 1 day (2 days before DLI; n = 3) or 3 days (days −4 to −2 before DLI; n = 2) (Table 1). Pentostatin was given as an IV bolus injection after infusion of 500 mL of saline solution, as reported previously [16]. Pretransplantation pentostatin (either 3×4 mg/m^2 or 6×4 mg/m^2 IV) administration was previously shown to prolong DLA-identical marrow engraftment in dogs given 1 Gy of TBI and posttransplantation MMF plus CSP [16]. The number of T cells in the DLIs was

Table 1. *Duration of Mixed Donor–Host Chimerism in Dogs Given Marrow Grafts from DLA-Identical Donors after 1 Gy TBI Followed (Study Group) or Not Followed (Historical Group) by Preemptive DLI Given 1 Month after Transplantation*

Dog No.	Nucleated Marrow Cells ($\times 10^8$/kg)	Pentostatin?	DLI: T Cells $\times 10^8$/kg (day after HCT)	Percent Donor Chimerism in PBMC before DLI	Duration of Mixed Chimerism, Among PBMC, in Weeks (final % of donor chimerism in PBMC)
Historical group					
E165	4.0	No	No	/	12 (0)
E166	4.0	No	No	/	10 (0)
E202	4.1	No	No	/	3 (0)
E204	4.0	No	No	/	3 (0)
E227	4.0	No	No	/	10 (0)
E228	4.0	No	No	/	10 (0)
G092†	3.6	No	No	/	9 (0)
G111†	6.3	No	No	/	11 (0)
G151†	3.7	No	No	/	3 (0)
G156†	3.8	No	No	/	9 (0)
G167†	4.2	No	No	/	9 (0)
Study group					
G335	6.3	No	1.0 (36)	40.0	>32 (46.5)
G376	2.4	No	2.0 (28)	8.8	15 (0)
G329	2.4	No	2.3 (39)	7.7	10 (0)
G444	5.4	No	2.6 (29)	29.3	>35 (5.0)
G468	4.0	4 mg/m^2	1.6 (38)	23.1	16 (0)
G509	3.8	4 mg/m^2	1.6 (30)	38.6	12 (0)
G541	3.5	4 mg/m^2	1.1 (30)	16.2	>33 (15.8)
G565	2.8	3 × 4 mg/m^2	1.7 (33)	9.9	7* (0)
G582	5.4	3 × 4 mg/m^2	1.4 (33)	14.8	12 (0)

*Eight weeks in the granulocyte fraction.
†These dogs were given CSP plus rapamycin instead of CSP plus MMF as postgrafting immunosuppression.

assessed by flow cytometry using a monoclonal antibody directed against canine CD3 (CA17.6F9, IgG_{2b}), as reported previously [15]; levels ranged from 1.0×10^8 to 2.6×10^8 T cells/kg (Table 1).

The presence of donor cells in PBMCs and granulocytes after HCT was assessed by fluorescent variable number tandem repeat polymerase chain reaction assays using an ABI Prism 310 Genetic Analyzer and Gene Scan 3.1 software (Applied BioSystems, Foster City, CA) [15]. The endpoint of the study was stable mixed donor–host hematopoietic chimerism beyond 30 weeks after HCT.

Data from dogs in this study were compared with those from a historical group of 11 dogs given DLA-identical marrow grafts after 1 Gy TBI and receiving postgrafting immunosuppression with either CSP and MMF or CSP and rapamycin [8,9].

The study was approved by the Institutional Animal Care and Use Committee at the Fred Hutchinson Cancer Research Center.

RESULTS

Engraftment, DLI, and Graft Rejection

Initial donor engraftment was documented in all dogs. Two of the 4 dogs given DLI only maintained stable mixed donor–host chimeras beyond 30 weeks after HCT, whereas 2 rejected their grafts, on weeks 10 and 15 (Table 1). We then investigated in 5 dogs whether host immunosuppression with pentostatin before DLI could allay the pending rejection by increasing the efficacy of DLI. One of the 5 dogs given pentostatin before DLI maintained a stable mixed donor–host chimera beyond 30 weeks, whereas the other 4 (including the 2 dogs given 3 doses of pentostatin) rejected their grafts, on weeks 8, 12, 12, and 16 after HCT (Table 1). Donor PBMC chimerism levels before DLI were 28.5% ± 11.9% in the 3 dogs with sustained engraftment, compared with 17.2% ± 11.9% in the 6 dogs with graft rejection (P = not significant [NS]). The absolute lymphocyte counts before DLI were 1203 ± 741 in the 3 dogs with sustained engraftment, versus 1990 ± 712 in the 6 dogs with graft rejection (P = *NS*).

Toxicity

Hematologic toxicities were generally mild (Figure 1). Median platelet, neutrophil, and lymphocyte nadirs were 32,000, 2077, and 396 cells/μL, respectively. Changes in lymphocyte counts were not statistically different between the dogs given and not given pentostatin (data not shown).

The main nonhematologic toxicity was gastrointestinal; effects included anorexia, diarrhea, and moderate weight loss, most likely due to MMF. In addition, 1 animal (G565) developed intussusception at day 26 after HCT that was successfully removed surgically. Intussusception is associated with the use of CSP in dogs.

Pentostatin and DLI were well tolerated. Graft-

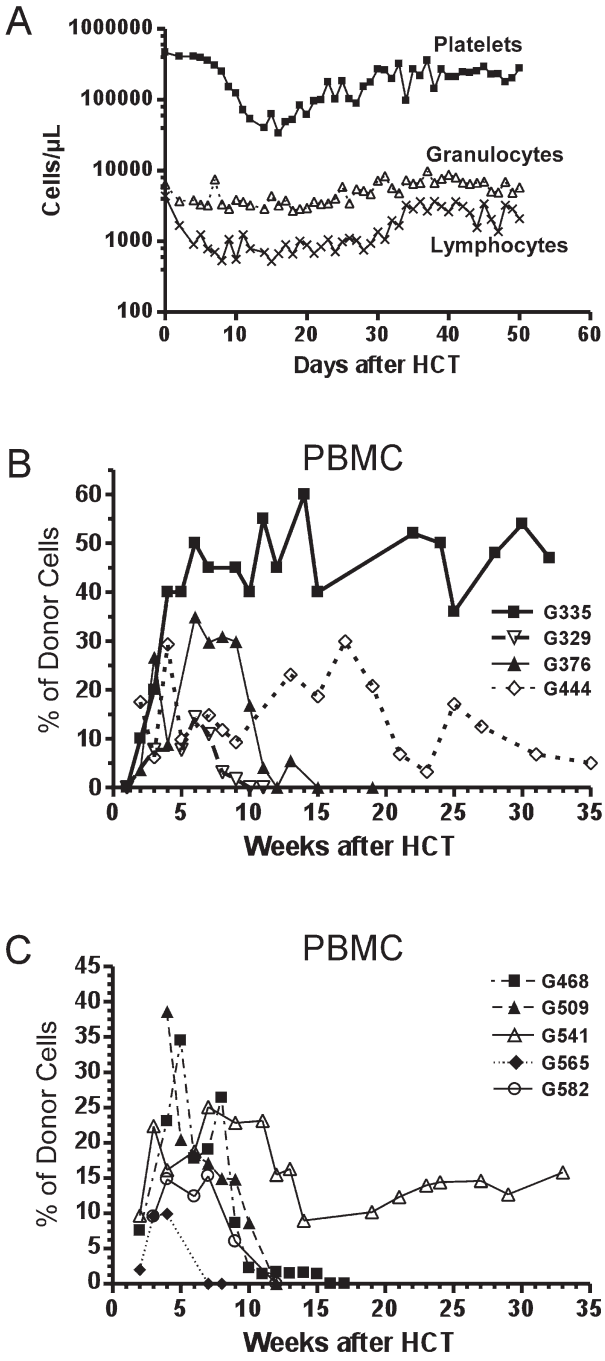

Figure 1. (A) Median peripheral blood changes in 9 dogs given 1 Gy of TBI before and MMF/CSP plus DLI (with or without preceding pentostatin) after marrow transplantation from DLA-identical donors; (B) PBMC chimerism in 4 dogs given 1 Gy of TBI before and MMF/CSP plus DLI after marrow transplantation from DLA-identical donors; (C) PBMC chimerism in 5 dogs given 1 Gy of TBI before and MMF/CSP, pentostatin plus DLI after marrow transplantation from DLA-identical donors.

versus-host disease (GVHD) was not observed in any dog.

Comparison of Graft Rejection with Historical Dogs

The numbers of nucleated marrow cells transplanted in present (2.4×10^8/kg–6.3×10^8/kg [median, 3.8×10^8/kg]) and historical dogs (3.6×10^8/kg-6.3×10^8/kg [median, 4.0×10^8/kg]) were comparable ($P = .3$). There was a statistical trend for decreased risk of rejection in dogs receiving higher numbers of nucleated marrow cells (analyzed as a continuous variable; $P = .07$). The 30-week probability of sustained donor engraftment was 33% in dogs given DLI, versus 0% in historical dogs ($P = .003$ in univariate analysis and $P = .002$ after adjusting for the number of nucleated marrow cells transplanted). After excluding the 5 historical dogs given CSP plus rapamycin as postgrafting immunosuppression, the P values were .03 and .02, respectively.

DISCUSSION

In contrast to cancer patients undergoing nonmyeloablative HCT, who most often received various amount of cytotoxic therapy before nonmyeloablative conditioning, dogs included in the current study did not receive any chemotherapy before conditioning to HCT, making them an ideal model for assessing ways to further improve donor engraftment. In a previous study, we observed stable mixed donor–host chimerism in 5 of 8 dogs given 1 Gy of TBI followed by DLA-identical marrow plus granulocyte-colony-stimulating factor–mobilized PBMC grafts (containing $1.9 \pm 0.5 \times 10^8$ T cells/kg), and in 1 of 7 dogs given 1 Gy of TBI followed by marrow plus CD3-depleted G-PBMCs [13], suggesting that the addition of donor T cells to the marrow grafts promoted sustained engraftment. Here we investigated whether infusion of additional donor T cells as late as 5 weeks after HCT, after discontinuation of immunosuppression, would promote stable engraftment in dogs given marrow grafts after 1 Gy of TBI.

Given in this manner, DLIs prevented graft rejection in only 2 of 4 dogs, suggesting that host T cells in the other 2 dogs were already sensitized against donor minor histocompatibility antigens when immunosuppression was discontinued and were capable of destroying both the marrow graft and the infused donor lymphocytes. The lack of uniform efficacy of DLI in the current canine model is consistent with observations made in human patients, where DLIs were often ineffective in preventing graft rejection in settings of low donor chimerism levels [17,18].

We next evaluated whether pentostatin, a potent immunosuppressive drug in both humans and dogs [16,19], could reduce the number of sensitized host lymphocytes sufficiently to set the stage for DLI to promote stable donor engraftment. This strategy met with little success, because only 1 of 5 dogs achieved stable mixed chimerism. Perhaps pentostatin affected not only sensitized host T cells, but also regulatory T cells [20], which have been implicated in the maintenance of stable mixed chimerism after nonmyeloablative conditioning [21,22].

Pentostatin and DLI appeared to be safe, and no acute GVHD was seen in dogs that achieved sustained engraftment. This was most likely related to the mixed donor–host T-cell chimerism with relatively low donor contributions, a condition associated with a low incidence of GVHD [6].

In conclusion, DLI was only moderately effective in allaying graft rejection in this model, even when preceded by host immunosuppression with pentostatin. The canine model might be useful in developing better strategies for preventing graft rejection in patients with low donor chimerism levels. These strategies should be aimed at specifically targeting activated (sensitized) T cells, without affecting regulatory T cells. Potential candidates include bortezomib, recently shown to specifically inhibit activated T cells [23], and radionuclide-conjugated monoclonal antibodies targeting the CD70 antigen [24].

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